

MANAGEMENT OF VASCULAR WILT OF LENTIL THROUGH HOST PLANT RESISTANCE, BIOLOGICAL CONTROL AGENTS AND CHEMICALS

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Abstract

The management of devastating lentil (*Lens culinaris* Medik.) wilt disease was investigated through evaluation of host plant resistance, biological control agents and seed treatment with different fungicides against a known most aggressive isolate i.e. FWL12 (KP297995) of *Fusarium oxysporum* f. sp. *lentis*. The *In vitro* screening of germplasm (23 advanced lines and cultivars) for host resistance by root dip method revealed five cultivars viz. Markaz-09, Masoor-86, Masoor-2006, Punjab Masoor-00518 and Punjab Masoor-09 resistant with 20 to 46.67% incidence, 4.44 to 12.95% severity index and 9.60 to 24.94% yield reduction compared with highly susceptible (100% incidence) local lentil line (NARC-08-1). The later line was treated with *Trichoderma* species as antagonists in pot experiment by drenching. The bio-control treatment revealed maximum positive effect of *T. harzianum* (26.7% incidence, 8.9% severity index and 16.27% yield reduction), followed by *T. viride* (66.7% incidence, 17.8% severity index and 31.13% yield reduction). On inoculated untreated control, the fungus produced the characteristic wilt symptoms and significantly caused increased severity index, incidence and decreased 100% yield. *In vitro* evaluation of four fungicides at five concentrations (10, 20, 30, 50 and 100 ppm) revealed maximum inhibition of the test fungus with benomyl (85.9%), followed by thiophanate methyl (81.2%). Determination of the efficacy of two best fungicides viz. benomyl and thiophanate methyl in reducing wilt infection through *In vivo* seed treatment of NARC-08-1 in previously inoculated potting mixture revealed 100% seed germination and suppressed wilt disease, the most effective being benomyl with 6.7% incidence, 1.5% wilt severity and 17.16% yield reduction compared to the control. The study concluded that the genetic diversity already present in lentil cultivars is an important source, which could be exploited for breeding wilt resistant lentil genotypes. Moreover, being seed and soil-borne, the disease could effectively be managed using the reported biological control agents and systemic fungicides in integrated disease management of lentil wilt.

Key words: *Fusarium oxysporum* f. sp. *lentis*, Lentil wilt, Host plant resistance, Biological control, Fungicides.

Introduction

Lentil (*Lens culinaris* Medik.) is an important pulse crop and the second major source of dietary proteins (25%) after soybeans in human and animal diet (Rahman *et al.*, 2010). In Pakistan, it is the extensively grown cool season legume crop next to chickpea in terms of quality and quantity (Khan *et al.*, 2001). The crop is infected by a number of fungal plant diseases and out of which vascular wilt is the most devastating caused by several *Fusarium* species. Globally, *Fusarium oxysporum* f. sp. *lentis* Vasudeva & Srinivasan is recognized the most important factor in reducing lentil production (Saxena, 1993; Erskine *et al.*, 2009). The wilt disease occurs in fields in patches and originates either at early (seedling) crop stage or at reproductive (adult plant) stage (Stoilova & Chavdarov, 2006).

Presently, lentil production in Pakistan is facing continuous decrease as a result of several biotic stresses including wilt (Subhani *et al.*, 2007). A number of management strategies aiming at controlling wilt disease are in practice. The cultural practices like crop rotation is common, however, it is not much effective because the pathogen is of seed or soil-borne nature and can survive in soil for extended period of time. Biological management and chemical seed treatment are considered to be the most effective in eradicating the inoculum present in seed and soil. Among biological control agents, *Trichoderma* species are considered the most effective against several fungal pathogens including *F.*

oxysporum (Sarhan *et al.*, 1999). These antagonists are saprophytic filamentous fungi, easily growing and produce conidia having long survival period in large quantities (Mohamed & Haggag, 2006). Under high inoculum conditions, suitable seed dressing fungicides are also most effective besides bio-control agents (Garkoti *et al.*, 2013).

Presence of high mutations and variations among the pathogen populations limit the effectiveness of natural resistance in the host plants against the pathogens (Nimbalkar *et al.*, 2006). Therefore, it is essential to determine the variability in the pathogen regarding its host plant resistance for a successful lentil breeding plan and replacing the low yielding and disease susceptible lentil varieties with those of high yielding and disease resistance ones. Considering these facts, this study was carried out to evaluate the available lentil germplasm, bio-control agents and fungicides against the disease in order to identify sources of resistance, effective bio-control agents and fungicidal seed treatment for the management of lentil wilt disease.

Materials and Methods

For the management of lentil wilt disease, three experiments viz., screening of available lentil germplasm against the pathogen, evaluation of bio-control agents and chemical fungicides were carried out at PMAS-Arid Agriculture University Rawalpindi, Pakistan during 2013 and 2014 cropping seasons.

Management through host plant resistance

Source and preparation of inoculum: A known most highly virulent isolate FWL12 (GenBank accession number KP297995) of *F. oxysporum* f. sp. *lentis* from lentil growing area of district Layyah (23°54'S; 21°55'E), Punjab, Pakistan was used for the study (Rafique *et al.*, 2015). For the inoculation of plants, the inoculum of the pathogen was prepared according to method of Taheri *et al.* (2010). The Erlenmeyer flasks (100 ml) containing 50 ml potato-dextrose broth were inoculated with mycelial agar disc (5 mm diameter) taken from pure culture of the isolate and shaken in rotary shaker at 120 rpm for three days. The spore suspension was adjusted to 1×10^7 conidia/ ml using haemocytometer.

Preparation of potting mixture: The potting mixture for pathogenicity test was prepared by sterilizing with formaldehyde (5%) prepared from 37% commercial formulation (Merck, Germany). The mixture was mixed thoroughly with prepared formaldehyde solution (100 ml/ kg of soil) and covered with polythene sheets with the edges properly air-sealed. After 2 days, the treated mixture was exposed to air to allow escape of fumes and the mixture was left exposed for about 4-5 days. After sterilization, the potting mixture was used for filling the pots.

Disease screening: A set of 23 lentil germplasm lines (17) and cultivars (6) obtained from National Agriculture Research Center (NARC), Islamabad, Pakistan were used for *In vitro* screening under screen house conditions after Taheri *et al.* (2010) with minor modification. Before planting, the lentil seeds were surface sterilized using 0.5% sodium hypochlorite for 2 minutes, rinsed in sterile water thrice and then germinated in plastic germinator trays filled with sterilized potting mixture (sand/farmyard manure, 1:1). After 15 days, the seedlings were uprooted carefully, dipped into the inoculum for about 10 minutes and then sown in plastic pots (5 seedlings per pot) containing sterilized potting mixture (sand/clay/farmyard manure, 1:1:1). The pots planted with non-inoculated seedlings but dipped in sterilized distilled water served as control. The pots were maintained under screen house and watered as required. The layout for the experiment was a completely randomized design (CRD).

Disease scoring: The disease score data recording was started from the 5th day after pathogen inoculation, which continued up to maturity. The recorded disease parameters included disease incidence, disease severity index and yield reduction. Disease incidence percentage was calculated using the formula as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity index percentage was determined using 0-9 rating scale proposed by Bayaa *et al.* (1995) with minor modification. Plants that showed infection type 0 were considered as immune (with 0% infection),

those with a score of 1-3 as resistant (1-25% plants wilted), with 4-6 as moderate (25-50%) and with 7-9 being susceptible (50% or more). The severity index percentage was then calculated by using the formula of Kranz (1988) as follows:

$$\text{Disease severity index (\%)} = \frac{\sum (a \times b)}{N \times Z} \times 100$$

where,

$\sum (a \times b)$ = Sum of the symptomatic plant and their corresponding scale value

N = Total number of plants per pot

Z = Highest scale value

Yield reduction was noted by harvesting the seeds and measuring the seed weight per 15 plants (five plants per three replications).

Management through bio-control agents: Two antagonistic species of *Trichoderma* viz., *T. harzianum* and *T. viride* obtained from Crop Diseases Research Institute (CDRI), NARC, Islamabad, were evaluated as bio-control agents against wilt. The conidial suspensions of both species were prepared in 1 litre sterilized distilled water using 5 mm mycelia disc from the margin of actively growing colonies. For inoculation, 40 ml suspensions were mixed separately using vortex mixture and adjusted to 5×10^8 conidia/ ml. The seeds of susceptible local lentil line viz., NARC-08-1 were used for the experiment. Five seeds per pot were sown in plastic pots containing sterilized potting mixture (sand: soil: FYM, 1:1:1) prepared as described above. After 15 days, the seedlings were inoculated with 60 ml spore suspension (1×10^7 conidia/ ml) of the virulent isolate and 40 ml suspensions of bio-control agents by drenching. After inoculation, pots were watered as required and maintained in screen house. The inoculated pots with no bio-control agents and un-inoculated pots with sterilized distilled water served as control and the experiment formed the CRD.

Disease parameters: Bio-control activity of both the microbes was measured by disease incidence, disease severity index and yield reduction on treated plants. Disease scoring was started upon appearance of wilt symptoms on un-inoculated control from 5th day and continued till maturity. Disease incidence, severity index and yield reduction were recorded as described above.

Management through fungicides

***In vitro* evaluation of fungicides:** Four fungicides including each of two non-systemic viz. dithane M-45 (mancozeb 80%WP) and captan (captan 50%WP) and two systemic viz. benomyl (benlate 50%WP) and thiophanate methyl (topsins-M 70%WP) were evaluated for their efficacy in petri plates (9 cm) using poison food technique (Nene & Thapliyal, 2000). Each test fungicide was checked at five concentrations viz. 10, 20, 30, 50 and 100 ppm. The experiment was conducted in two factor factorial design.

The required weighed quantity of each test fungicide was amended to the autoclaved malt extract agar (MEA) medium to obtain required concentration and around 20 ml of both amended and non-amended media was poured in 9 cm plates. Plates without amended fungicide were used as control. Each amended plate was then inoculated with 5 mm mycelial agar disc from the edge of freshly grown culture plate using sterile cork borer. The inoculated media plates were then incubated at 25±2°C for about 7 days. After incubation, when control plates were filled completely with mycelial growth, data on radial growth was calculated. The efficacy of fungicides was expressed as percent mycelial growth inhibition over control and was calculated by using the formula (Vincent, 1947):

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

where,

C = Growth of test fungus in control (cm)

T = Growth of test fungus in treatment (cm)

In vivo fungicidal seed treatment: The fungicides viz. benomyl and thiophanate methyl exhibiting maximum mycelial *In vitro* inhibition were tested *In vivo* by seed treatment prior to sowing in plastic pots under screen house conditions. The seeds of the susceptible local lentil line NARC-08-1 were used for the experiment and treated with both test fungicides (2 gm/ kg seed) by dressing with wet slurry formulation. Sterilized potting mixture (sand: soil: FYM, 1:1:1) was again used for planting the seeds (5 per pot) in plastic pots. Before planting, the mixture was inoculated with the spore suspension (1×10^7 conidia/ ml). After seed sowing, the pots were watered as required and maintained in screen house. The inoculated pot with untreated seeds and uninoculated pot with treated seeds served as control and this experiment also formed CRD. Data on seed germination, disease incidence, severity index and yield reduction were recorded from germination till maturity.

Statistical data analysis: All the experiments were conducted in triplicate per treatment and fungicide concentration. The mean data on the disease parameters was taken, analyzed statistically using software program SPSS. Least significance difference (LSD) was measured at $p = 0.05$ for all data variables to compare treatments means.

Results

Management through host plant resistance: Screening of available lentil germplasm was conducted in pot experiment employing root dip method and the data was analyzed based on disease parameters (disease incidence, severity index and yield reduction) starting at varied days mostly 15-20 days after inoculation. Initially, symptoms started with the leaf distortion and some chlorosis. Later, more chlorosis on foliage, necrosis and plant stunting was

observed. The susceptible plants showed wilted growth with vascular discoloration and ultimately collapsed. Based on these symptoms and the assessment of wilt incidence and severity, screened lines and cultivars were characterized into three groups of infection types as resistant, intermediate or moderate and susceptible using the 0-9 rating scale. None of the line or cultivar was found immune against the disease. The data on percent disease incidence, severity index and yield reduction are presented in Table 1. Overall, disease incidence ranged from 20 to 100%, disease severity index ranged from 4.44 to 100%, while yield reduction from 9.60 to 100% among the germplasm tested.

The screening results showed that among the 23 lentil germplasm lines and cultivars, five cultivars viz. Markaz-09, Masoor-86, Masoor-2006, Punjab Masoor-00518 and Punjab Masoor-09 were found significantly resistant against the disease and scored between 1-3 on the rating scale. The wilt incidence and severity index in resistant cultivars ranged from 20 to 46.67% and 4.44 to 12.59%, respectively, with 9.60 to 24.94% reduction in seed yield. Two lines viz. BL-2 and NL-1 gave moderate reaction falling between 4-5 scale range and showed 40 and 46.67% incidence, 22.22 and 30.37% severity index, whereas, yield reduction was 41.2 and 61.94%. The rest of the lines including Mansehra-89 bold seeded, NL-2, NL-3, NARC-08-2, NARC-11-1, NARC-11-2, NARC-11-3, NARC-06-1, 08504, 08505, 09506, 01505, 03501, 04533, 06513 and NARC-08-1 were found susceptible on 7-9 scale range and indicated 100% incidence and 60.74 to 100% severity index. These susceptible lines were found dead completely before maturity and resulted in 100% yield reduction except one line viz. NL-3 with 77.90% reduction produced few seeds which were found shriveled. The data showed a great variation in seed yield among the tested lines and cultivars and proved that wilt disease significantly affects the grain yield and its quality suggesting that higher the wilt incidence and severity, lower will be the seed quantity and quality.

Management through bio-control agents: The biological control agents viz., *T. harzianum* and *T. viride* were used as soil treatments for the management of *Fusarium* lentil wilt. To analyze the influence of both the microbes on pathogen, disease parameters such as disease incidence, severity index and yield reduction were scrutinized. Results revealed the effectiveness of both the species; however, maximum control was recorded with *T. harzianum* as compared to *T. viride* (Fig. 1). The treatment with *T. harzianum* showed significant difference with control plants. The disease severity index with *T. harzianum* was found to be 8.9%, while disease incidence was 26.7% with 16.27% yield reduction. The inoculated control was observed with 100% severity, incidence and yield reduction whereas, un-inoculated control was found with 0% severity and incidence. On the other hand, treatment with *T. viride* showed 17.8% severity index and 66.7% incidence with the yield reduction of 31.13%. This data showed that *T. harzianum* was much effective treatment for biological control of *Fusarium* wilt.

Table 1. *In vitro* screening of lentil germplasm against *F. oxysporum* f. sp. *lentis*.

Sr. no.	Tested germplasm	Disease severity index*		Disease incidence		Yield reduction		Infection type
		(%)		(%)		(%)		
		Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	
1	Markaz-09	5.92 ^j	0 ^j	26.67 ^c	0 ^d	24.94 ^c	0 ^g	R**
2	Masoor-86	4.44 ^j	0 ^j	20 ^c	0 ^d	20.63 ^e	0 ^g	R
3	Masoor-2006	5.92 ^j	0 ^j	26.67 ^c	0 ^d	11.69 ^f	0 ^g	R
4	Punjab Masoor-00518	12.59 ⁱ	0 ^j	46.67 ^b	0 ^d	12.72 ^f	0 ^g	R
5	Punjab Masoor-09	4.44 ^j	0 ^j	20 ^c	0 ^d	9.60 ^f	0 ^g	R
6	BL-2	22.22 ^h	0 ^j	40 ^b	0 ^d	41.2 ^d	0 ^g	M***
7	NL-1	30.37 ^g	0 ^j	46.67 ^b	0 ^d	61.94 ^c	0 ^g	M
8	Mansehra-89 Bold seeded	99.26 ^{ab}	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S****
9	NL-2	91.85 ^{cd}	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
10	NL-3	60.74 ^f	0 ^j	100 ^a	0 ^d	77.90 ^b	0 ^g	S
11	NARC-08-2	93.33 ^{bcd}	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
12	NARC-11-1	99.26 ^{ab}	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
13	NARC-11-2	100 ^a	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
14	NARC-11-3	100 ^a	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
15	NARC-06-1	97.04 ^{abc}	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
16	08504	89.62 ^d	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
17	08505	99.26 ^{ab}	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
18	09506	94.81 ^{abcd}	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
19	01505	100 ^a	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
20	03501	81.48 ^e	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
21	04533	90.36 ^d	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
22	06513	100 ^a	0 ^j	100 ^a	0 ^d	100 ^a	0 ^g	S
23	NARC-08-1 (Susceptible check)	100 ^a	0 ^j	100 ^d	0 ^d	100 ^a	0 ^g	S
LSD value at $\alpha=0.05$		5.93		8.73		4.40		-

At $\alpha=0.05$ level of significance means sharing same letters are non-significant

Data based on mean of three replications

*Disease severity percentage on the basis of 0-9 scale (Bayaa *et al.*, 1995), where 1-3 = **resistant (1-25% plants wilted), 4-6 = ***moderate (25-50%) and 7-9 = ****susceptible (50% or more)

Table 2. *In vitro* effect of fungicides at different concentrations on percent growth inhibition of *F. oxysporum* f. sp. *lentis*.

Fungicide	Mycelial growth inhibition at fungicide concentration (ppm) (%)					
	10ppm	20ppm	30ppm	50ppm	100ppm	Mean
Dithane M-45	40.0 ⁿ	60.4 ^k	63.0 ^j	70.4 ^h	77.8 ⁱ	62.3 ^b
Captan	42.2 ^m	64.1 ^j	74.4 ^g	78.1 ^f	80.4 ^e	67.8 ^{ab}
Benomyl	46.7 ^l	71.1 ^h	86.7 ^c	88.5 ^b	90.0 ^a	76.6 ^a
Thiophanate methyl	43.0 ^m	68.9 ⁱ	81.1 ^e	83.7 ^d	88.1 ^b	73.0 ^{ab}
Control	0 ^o	0 ^o	0 ^o	0 ^o	0 ^o	0 ^c
Mean	43.0 ^d	66.1 ^c	76.3 ^b	80.2 ^{ab}	84.1 ^a	69.9

LSD value at $\alpha=0.05$: Fungicide = 11.53; Concentration = 5.15; Interaction = 1.35

At $\alpha=0.05$ level of significance means sharing same letters are non-significant

The results confirmed that the inoculation of conidial suspension of *T. harzianum* significantly ($p \leq 0.05$) decreased the lentil wilt disease (73.33% wilted lentil plants) compared to inoculated control. This decrease in wilt incidence in contrast to inoculated control may be attributed to an increase in the population density of bio-control agent in the potting mixture.

Management through fungicides

***In vitro* evaluation of fungicides:** Fungicidal treatments against the fungal pathogen indicated that all the tested fungicides checked the mycelial growth of the pathogen at variable rate with the mean reduction of 69.9%. Out of the four fungicides, benomyl and thiophanate methyl had a marked significant inhibitory effect on the mycelial growth of the pathogen (Table 2). Fungicides differentially limited the colony growth of the pathogen, where most considerable growth check was indicated by

benomyl (mean 76.6%), followed by thiophanate methyl (73.0%), captan (67.8%) and dithane M-45 (62.3%).

Evaluation studies at five concentrations of the fungicides revealed that the inhibition of mycelial growth increased with the increase in concentration compared to the control (Table 2). At the lowest 10 ppm concentration, the highest inhibition was observed in the case of benomyl (46.7%) that showed 4.8 ± 0.10 cm growth, followed by thiophanate methyl (43% inhibition; 5.1 ± 0.06 cm growth), captan (42.2%; 5.2 ± 0.0 cm) and dithane M-45 (40%; 5.4 ± 0.10 cm). Similarly, at 20, 30 and 50 ppm concentrations, each fungicide showed decreased fungal growth that continued in reduction with the increase in concentration. The benomyl was superior in maximum inhibition and minimum radial growth at all three concentrations (71.1, 86.7, 88.5%; 2.6 ± 0.0 , 1.2 ± 0.0 , 1.0 ± 0.06 cm), followed by thiophanate methyl (68.9, 81.1, 83.7%; 2.8 ± 0.0 , 1.7 ± 0.10 , 1.5 ± 0.06 cm), captan (64.1, 74.4, 78.1%; 3.2 ± 0.06 , 2.3 ± 0.10 , 2.0 ± 0.06 cm) and

dithane M-45 (60.4, 63.0, 70.4%; 3.6±0.06, 3.3±0.12, 2.7±0.12 cm) with inferior response. Best fungus control was observed at the highest fungicidal concentration (100 ppm) at which, all the fungicides greatly inhibited the growth of fungal pathogen. At this concentration, benomyl caused maximum growth inhibition and produced minimum growth i.e. 90% and 0.9±0.10 cm. Other fungicides including thiophanate methyl (88.1%; 1.1±0.12 cm) and captan (80.4%; 1.8±0.06 cm) showed the intermediate reduction response, while dithane M-45 (77.8%; 2.0±0.0 cm) showed the least inhibitory result on the mycelial growth.

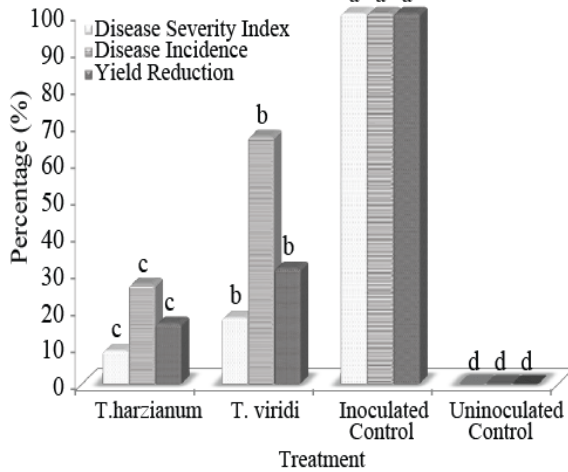


Fig. 1. Effect of bio-control agents on disease severity index, disease incidence and grain yield reduction caused by *F. oxysporum* f. sp. *lentis* in a green house experiment. Different letters indicated on bars represent significant differences in wilt severity, incidence and yield reduction values ($p \leq 0.05$). LSD = 4.18 severity, 15.37 incidence, 3.33 yield reduction.

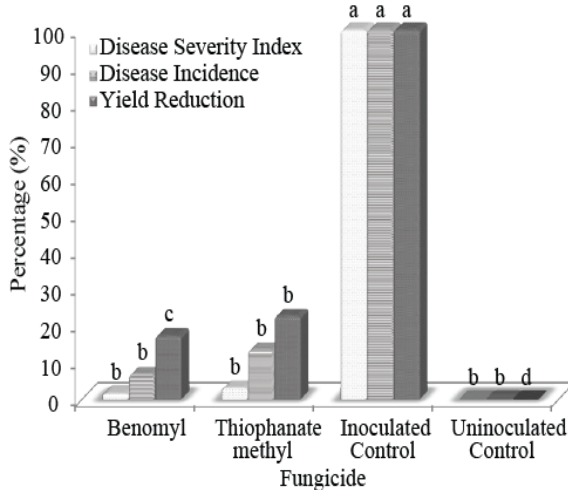


Fig. 2. Effect of fungicidal seed treatment on disease severity index, disease incidence and grain yield reduction caused by *F. oxysporum* f. sp. *lentis* in a green house experiment. Different letters indicated on bars represent significant differences in wilt severity, incidence and yield reduction values ($p \leq 0.05$). LSD = 5.53 severity, 24.3 incidence, 1.77 yield reduction.

In vivo fungicidal seed treatment: To determine the influence of most effective fungicides (benomyl and thiophanate methyl) on wilt through seed treatment, disease parameters including seed germination, disease incidence, disease severity index and yield reduction were scrutinized. Results revealed the significant effect of both the fungicidal treatments ($p \leq 0.05$) with 100% seed germination and efficient control of the disease compared to the inoculated untreated check (Fig. 2). Reduced disease incidence, severity index and yield reduction was observed compared to the inoculated untreated control, though no improvement was observed in the yield component compared to un-inoculated untreated check. Benomyl was proved superior in the experiment that resulted in 1.5% disease severity index, 6.7% incidence and 17.16% yield reduction compared to thiophanate methyl (3% severity index, 13.3% incidence and 22.47% yield reduction). The results of the study showed that systemic fungicides (benomyl and thiophanate methyl) are more effective (both *In vitro* and *In vivo*) in managing wilt disease compared to the non-systemic fungicides (dithane M-45 and captan) suggesting that seed and soil-borne inoculum can be removed effectively by using these chemicals.

Discussion

The pathogenic fungus *F. oxysporum* f. sp. *lentis* is supposed to be responsible for severe disease damage under hot and dry weather conditions (Bayaa & Erskine, 1998) with the temperature ranges of 22–25°C, suitable for disease development, thus causing huge losses in areas with such conditions (Mohammadi *et al.*, 2011). In the present study, a significant variation in resistance against the wilt disease was observed among the tested germplasm under controlled screen house conditions. The pathogenic virulence showed variable effects on different germplasm tested viz. resistant, moderate and susceptible reactions. This observation suggests that the presence of similar environmental condition and amount of inoculum provided, the genetic makeup of the plant also affects the resistance reaction of the plants towards the fungus (Mohammadi *et al.*, 2012).

The root dip method employed in this study proved to be efficient and reproducible in producing wilt disease and symptoms as demonstrated by Taheri *et al.* (2010). Disease parameters including percent wilt incidence and percent severity index were noted based on typical wilt symptoms (Bowers & Locke, 2000). The variation in incubation period occurred depending upon the resistance reaction offered by different germplasm against the pathogen. Based on a modified disease rating scale described by Bayaa *et al.* (1995), the screened lines and cultivars were grouped into three categories as resistant, moderate and susceptible, thus helped in the identification of resistant sources. The susceptible check lentil line NARC-08-1 showed clear wilt symptoms that were visible after 15 days of inoculation, which continuously increased and resulted in complete death later in the season. This was useful

in recording the onset of disease and greatly helped in characterizing the other tested germplasm accurately. Significant difference was observed in disease reaction and parameters among the germplasm tested as shown in Table 1. Resistance identified in five cultivars (Markaz-09, Masoor-86, Masoor-2006, Punjab Masoor-00518 and Punjab Masoor-09) suggests that these are an important source of genetic diversity to be exploited in lentil breeding programs against the disease. The total yield reduction observed in susceptible lines proved the deterioration of the quantity and quality of seed by the wilt disease.

Bio-control is the best and effective substitute, especially against soil-borne pathogens such as *Fusarium* species. This method offers advantages such as environment friendly, cost effective and extended plant protection (Gohel *et al.*, 2007). Among several antagonists used for biological management, *Trichoderma* species are used extensively as bio-control agents against soil and seed-borne diseases such as *Fusarium* wilt (Etebarian, 2006). In the present study, two species of *Trichoderma* were employed against highly virulent isolate of *Fusarium* responsible for lentil wilt. The results of the treatments suggested that both the microbes have the ability to reduce the disease damage, however, *T. harzianum* was highly efficient in controlling wilt disease and reducing severity of disease (8.9%) when applied as a soil drench as described by Jager *et al.* (1991). Similar results were reported by Dolatabadi *et al.* (2012), who observed reduced disease severity with increased plant height with the combination of *T. harzianum* + *S. vermifera*. Recently, Kumar *et al.* (2013) observed significant reduction in incidence and maximum grain yield in field trials against lentil wilt with *T. harzianum* + *Pseudomonas fluorescense*. Likewise, Akrami *et al.* (2011) evaluated three isolates of *Trichoderma* viz. T1 (*T. harzianum*), T2 (*T. asperellum*) and T3 (*T. virens*) alone and in combination against *Fusarium* rot of lentil. The green house experiment showed more effectiveness of isolates T1 and T2 isolates and their combination as compared to other treatments. Disease severity was found to be reduced ranging from 20 to 44%, while dry weight increased from 23 to 52%. Various other studies have also reported disease control using *Trichoderma* species, such as Poddar *et al.* (2004) reported decreased chickpea wilt incidence with isolate of *T. harzianum*. Correspondingly, Siddiqui & Singh (2004) found maximum plant growth, increased transpiration and decreased wilt disease index caused by *F. oxysporum* f. sp. *ciceris* through treatment with *T. harzianum*. Similarly, reduced wilt disease incidence in chickpea using *Trichoderma* species was later reported by Dubey *et al.* (2006). In a recent study, Shafique *et al.* (2015) found effective results with *T. harzianum* against *F. oxysporum* in *Vernonia* amended soil. Ghahfarokhi and Goltapeh (2010) have also reported *Trichoderma* species, *P. indica* and *S. vermifera* as the most effective bio-control agents against take-all diseases of wheat caused by *Gaeumannomyces graminis* var. *tritici*.

The management using fungicides revealed that systemic fungicides found to be superior to non-systemic fungicides in inhibiting the fungal mycelial growth in plates as well as in pot seed treatment. Benomyl (76.6%) showed the most positive inhibition results against the pathogen followed by thiophanate methyl (73.0%). Non-systemic fungicides viz. captan (67.8%) and dithane M-45 (62.3%) were the least efficient in reducing the fungal growth compared to the systemic fungicides. On the contrary, Kasyap *et al.* (2008) found much reduced fungal growth with captan (88.3%). Increased mycelial inhibition was observed with increased concentration of the fungicides compared with the control and the best fungus control was observed at highest fungicidal concentration (100 ppm). All the fungicides greatly inhibited the growth of the fungus at this concentration with the maximum reduction obtained with benomyl. In a similar study, Sharma *et al.* (2002) found no mycelial growth i.e. 100% inhibition of *F. oxysporum* f. sp. *lini* (linseed wilt) with benomyl at 500 ppm concentration. Later, Luz *et al.* (2007) also found benomyl with highest inhibition of fungus along with carbendazim and captan. Likewise, thiophanate methyl was second most effective at 100 ppm concentration. Similar results were reported by Maitlo *et al.* (2014) who found high efficacy of thiophanate methyl at 100, 1000 and 10000 ppm concentrations with complete suppression of colony growth of *F. oxysporum* f. sp. *ciceris*. At all concentrations checked in the study, dithane M-45 showed the least 77.8% inhibitory response with 2.0±0.0 cm radial growth. Comparatively, Singh *et al.* (2010) found 66% inhibition with 28 mm diameter at 200 ppm concentration of dithane M-45. Also, De *et al.* (2003) reported complete inhibition of growth of the pathogen by dithane M-45 i.e. 0.25%. Similarly, Dabbas *et al.* (2008) found complete inhibition of *F. oxysporum* f. sp. *pisi* at 200 ppm concentration.

In vivo seed treatment had positive effect on germination of all seeds used and thus, resulted in 100% germination producing healthy and vigor seedlings. Benomyl significantly controlled the wilt disease followed by thiophanate methyl as compared to the untreated control (Fig. 2). The treatments were efficient in reducing the percent disease severity index and incidence measured on a 0-9 scale (Bayaa *et al.*, 1995) compared to the inoculated check. However, the fungicidal treatment did not significantly increase the yield component compared to un-inoculated check and produced varied reduction percentage in yield. The results based on disease parameters (6.7% incidence, 1.5% severity and 17.16% yield reduction) showed that benomyl was proved superior compared to thiophanate methyl. Similar results were also reported by Garkoti *et al.* (2013) who observed benomyl with reduced wilt incidence (1.0%).

Conclusion

The lentil wilt caused by *F. oxysporum* f. sp. *lentis* is one of the significant production constraints in Pakistan and there are very limited resistance sources and control options available against the disease. The findings of this study clearly indicated effective bio-control agents, chemical seed treatment and available

resistant germplasm against this disease. The five wilt resistant cultivars viz. Markaz-09, Masoor-86, Masoor-2006, Punjab Masoor-00518 and Punjab Masoor-09 can be recommended for increasing cultivation by the farmers or can be utilized in lentil breeding programs for developing high yielding wilt resistant lentil genotypes. Soil application with bio-control agent like *T. harzianum* and seed treatment with systemic fungicides benomyl and thiophanate methyl further provided the most effective management options that can be used by the farmers. Following this approach, seed as well as soil-borne pathogen inoculum can possibly be eliminated. Moreover, the information regarding the application of the reported fungicides and bio-control agent can be used as an integrated disease management of lentil wilt.

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References

- Akrami, M., H. Golzary and M. Ahmadzadeh. 2011. Evaluation of different combinations of *Trichoderma* species for controlling *Fusarium* rot of lentil. *Afr. J. Biotechnol.*, 10: 2653-2658.
- Bayaa, B. and W. Erskine. 1998. Diseases of lentil. In: *Pathology of Food and Pasture Legumes*. (Eds.): Allen, D.J. and J.M. Lenne. CAB International, Wallingford, Oxon, UK, pp. 423-471.
- Bayaa, B., W. Erskine and A. Hamdi. 1995. Evaluation of a wild lentil collection for resistance to vascular wilt. *Genet. Resour. Crop Evol.*, 42: 231-235.
- Bowers, J.H. and J.C. Locke. 2000. Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the greenhouse. *Plant Dis.*, 84: 300-305.
- Dabbas, M., J. Srivastava and M. Rai. 2008. IDM for wilt disease of table pea. *Ann. Plant Protect. Sci.*, 16: 156-158.
- De, R.K., R. Dwivedi and U. Narain. 2003. Biological control of lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis*. *Ann. Plant Protect. Sci.*, 11: 46-52.
- Dolatabadi, H.K., E.M. Goltapeh, N. Mohammadi, M. Rabiey, N. Rohani and A. Varma. 2012. Biocontrol potential of root endophytic fungi and *Trichoderma* species against *Fusarium* wilt of lentil under *In vitro* and greenhouse conditions. *J. Agric. Sci. Technol.*, 14: 407-420.
- Dubey, S.C., M. Suresh and B. Singh. 2006. Evaluation of *Trichoderma* Species against *Fusarium oxysporum* f. sp. *ciceris*, for integrated management of chickpea (*Fusarium oxysporum* f. sp. *ciceris*). *Indian J. Agr. Sci.*, 74: 346-348.
- Erskine, W., F. Muehlbauer, A. Sarker and B. Sharma. 2009. *The Lentil: Botany, Production and Uses*. CAB International, Wallingford, UK.
- Etebarian, H. 2006. Evaluation of *Trichoderma* isolates for biological control of charcoal coal stem rot in melon caused by *Macrophomina phaseolina*. *J. Agric. Sci. Technol.*, 8: 243-250.
- Garkoti, A., A. Kumar and H. Tripathi. 2013. Management of *Fusarium* wilt of lentil through fungicides. *J. Mycol. Plant Pathol.*, 43: 333-335.
- Ghahfarokhi, R.M. and M.E. Goltapeh. 2010. Potential of the root endophytic fungus *Piriformosporandica*, *Sebacina vermifera* and *Trichoderma* species in biocontrol of take-all disease of wheat *Gaeumannomyces graminis* var. *tritici* *In vitro*. *Int. J. Agric. Technol.*, 6: 11-18.
- Gohel, V., V. Maisuria and H. Chhatpar. 2007. Utilization of various chitinous sources for production of mycolytic enzymes by *Pantoeadispora* in bench-top fermenter. *Enzyme Microb. Tech.*, 40: 1608-1614.
- Jager, G., H. Velvis, J.G. Lamers, A. Mulder and J. Roosjen. 1991. Control of *Rhizoctonia solani* in potato by biological, chemical and integrated measures. *Potato Res.*, 34: 269-284.
- Kasyap, A., P. Tiwari, C. Khare and V. Thrimurthy. 2008. Evaluation of varieties and fungicides against anthracnose and fruit rot of chilli. *Ann. Plant Protect. Sci.*, 16: 159-161.
- Khan, A., M. Rahim and A. Khan. 2001. Performance of exotic lentil varieties under rainfed conditions in Mingora (NWFP) Pakistan. *Online J. Biol. Sci.*, 1: 343-344.
- Kranz, J. 1988. Measuring plant disease. In: *Experimental Techniques in Plant Disease Epidemiology*. (Eds.): Kranz, J. and J. Rotem J, Springer, The Netherlands, pp. 35-50.
- Kumar, V., A. Garkoti and H. Tripathi. 2013. Management of vascular wilt of lentil through biocontrol agents and organic amendments in Tarai Area of Uttarakhand State. *The Bioscan*, 8: 575-577.
- Luz, C., M.C.B. Netto and L.F.N. Rocha. 2007. *In vitro* susceptibility to fungicides by invertebrate-pathogenic and saprobic fungi. *Mycopathologia*, 164: 39-47.
- Maitlo, S.A., R.N. Syed, M.A. Rustamani, R.D. Khuhro and A.M. Lodhi. 2014. Comparative efficacy of different fungicides against *Fusarium* wilt of chickpea (*Cicer arietinum* L.). *Pak. J. Bot.*, 46: 2305-2312.
- Mohamed, H.A.L.A. and W.M. Haggag. 2006. Biocontrol potential of salinity tolerant mutants of *Trichoderma harzianum* against *Fusarium oxysporum*. *Braz. J. Microbiol.*, 37: 181-191.
- Mohammadi, N., E. Goltapeh, A. Babaie-Ahari and H. Puralibaba. 2011. Pathogenic and genetic characterization of Iranian isolates of *Fusarium oxysporum* f. sp. *lentis* by ISSR analysis. *Int. J. Agric. Technol.*, 7: 63-72.
- Mohammadi, N., H. Puralibaba, E.M. Goltapeh, A.B. Ahari and B.P. Sardrood. 2012. Advanced lentil lines screened for resistance to *Fusarium oxysporum* f. sp. *lentis* under greenhouse and field conditions. *Phytoparasitica*, 40: 69-76.
- Nene, Y. and P. Thapliyal. 2000. Poisoned food technique. In: *Fungicides in Plant Disease Control*. (3rd Ed) Oxford & IBH Publishing Company, New Delhi, India.
- Nimbalkar, S.B., A.M. Harsulkar, A.P. Giri, M.N. Sainani, V. Franceschi and V.S. Gupta. 2006. Differentially expressed gene transcripts in roots of resistant and susceptible chickpea plant (*Cicer arietinum* L.) upon *Fusarium oxysporum* infection. *Physiol. Mol. Plant Pathol.*, 68: 176-188.
- Poddar, R., D. Singh and S. Dubey. 2004. Integrated application of *Trichoderma harzianum* mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*). *Indian J. Agr. Sci.*, 74: 346-348.
- Rafique, K., C.A. Rauf, F. Naz and G. Shabbir. 2015. DNA sequence analysis, morphology and pathogenicity of *Fusarium oxysporum* f. sp. *lentis* isolates inciting lentil wilt in Pakistan. *Int. J. Biosci.*, 7: 74-91.
- Rahman, T., A. Ahmed, M. Islam and M. Hosen. 2010. Physiological Study and both *In vitro* and *In vivo* antifungal activities against *Stemphylium botryosum* causing *Stemphylium* blight disease in lentil (*Lens culinaris*). *Plant Pathol. J.*, 9: 179-187.

- Sarhan, M., S. Ezzat, M. Tohamy, A.F. El-Essawy and E.S. Abd-Allah. 1999. Application of *Trichoderma hamatum* as a biocontroller against tomato wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Egypt. J. Microbiol.*, 34: 347-376.
- Saxena, M.C. 1993. The challenge of developing biotic and abiotic stress resistance in cool-season food legumes. In: (Eds.): Singh, K.B. and M.C. Saxena. *Breeding for stress tolerance in cool-season food legumes*. John Wiley & Sons, Chichester, UK, pp. 3-14.
- Shafique, H.A., R. Noreen, V. Sultana, J. Ara and S. Ehteshamul-Haque. 2015. Effect of endophytic *Pseudomonas aeruginosa* and *Trichoderma harzianum* on soil-borne diseases, mycorrhizae and induction of systemic resistance in okra grown in soil amended with *Vernonia anthelmintica* (L.) seed's powder. *Pak. J. Bot.*, 47: 2421-2426.
- Sharma, R., B. Singh, M. Thakur and K. Verma. 2002. Chemical management of linseed wilt caused by *Fusarium oxysporum* f. sp. *lini*. *Ann. Plant Protect. Sci.*, 10: 390-391.
- Siddiqui, Z. and L. Singh. 2004. Effects of soil inoculants on the growth, transpiration and wilt disease of chickpea. *J. Plant Dis. Protect.*, 111: 151-157.
- Singh, V.K., P. Naresh, S. Biswas and G.P. Singh. 2010. Efficacy of fungicides for management of wilt disease of lentil caused by *Fusarium oxysporum* f. sp. *lentis*. *Ann. Plant Protect. Sci.*, 18: 411-414.
- Stoilova, T. and P. Chavdarov. 2006. Evaluation of lentil germplasm for disease resistance to *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lentis*). *J. Cent. Eur. Agric.*, 7: 121-126.
- Subhani, G.M., N. Muhammad and A.R. Malhi. 2007. Status and scope of lentil production in Punjab, Pakistan. In: *Proceedings of International Conference: Achieving Sustainable Pulses Production in Pakistan, from 20-22, 2007*. Agricultural Foundation of Pakistan, pp.73-78.
- Taheri, N., M. Rastegar, B. Jafarpour, A. Bagheri and V. Jahanbaghsh. 2010. Pathogenic and genetic characterization of *Fusarium oxysporum* f. sp. *lentis* by RAPD and IGS analysis in Khorasan province. *World Appl. Sci. J.*, 9: 239-244.
- Vincent, J. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159: 850.

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